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RESEARCH ARTICLE

The feeding habits of the endemic Remo flounder, *Oncopterus darwinii* (Actinopterygii: Pleuronectidae), in an exposed sandy beach's surf zone in southern Brazil

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ABSTRACT. The Remo flounder, *Oncopterus darwinii* Steindachner, 1874 is a small Pleuronectidae (~ 250 mm TL) distributed in the warm-temperate zone from Santa Catarina (28°S – Brazil) to the Gulf of San Matías (42°S – Argentina). We studied the occurrence and diet of juveniles of this flatfish (85% < 100 mm TL) for one year (August 2009 to July 2010) in a southern Brazilian sandy beach. Individuals were much more common in spring and early summer than in the other seasons. Of the 758 individuals analyzed, only 69 (9.1%) were found with empty stomachs. The Remo flounder fed predominantly on small crustaceans (gammarid amphipods, mysids and the hipiidae *Emerita brasiliensis* Schmitt, 1935) and no seasonal differences were found in the composition of the diet of this flatfish. However, variations in the Index of Relative Importance (IRI) were found among seasons, which most likely can be explained by a decrease in the availability of favored food items throughout the year. The pattern of distribution of *O. darwinii* indicates that southern Brazilian sandy beaches are used as nursery ground for this species.

KEY WORDS. Feeding strategy, flatfish, marine fish, southwestern Atlantic Ocean.

INTRODUCTION

Several juvenile fish species use coastal areas (mainly sandy beaches and estuaries) as nurseries because these areas generally have higher productivity, offer lots of food and shelter from predators, and have favourable temperatures, all of which decrease the risk of mortality in the early life stages (Lasiak 1981, Cabral et al. 2002, Potter et al. 2013). These favourable environments have a high concentration of juveniles and this can result in inter- and intraspecific competition for food (Steele and Edwards 1970, Van der Veer and Witte 1993). Flatfish are no exception: juveniles of many species concentrate inside these nursery areas (Rountree and Able 1992, Burke 1995) and adults can be found at greater depths (Jones 1973, Wyche and Shackley 1986, Beyst et al. 1999, Nissling et al. 2007).

Flatfish species have a broad global distribution and great commercial and ecological importance (Gibson et al. 2015). Most flatfish species are benthic predators and are normally associated with sandy substrates. They have the ability to bury themselves in the sand, a camouflage technique that helps to protect them (Gibson and Robb 1992, Guedes and Araújo 2008, Gibson et al. 2015). *Oncopterus darwinii* Steindachner, 1874, the Remo flounder, is the only species that belongs to the family Pleuronectidae along the subtropical region of the South West Atlantic coast. It is endemic from that region and is distributed between Santa Catarina (Brazil – 28°S) to the Gulf of San Matías (Argentina – 42°S) (Figueiredo and Menezes 2000). Recently, a study by Bovcon et al. (2011) reported a southward expansion of *O. darwinii* to Central Patagonian waters (43–47°S), probably associated with the prevalence of warmer water temperatures

there (climate change). There is little information in the literature about where the adults live, but according to some records from fishery landings in the coast of Argentina and Fishbase.org, they can be found deep in the water 20–80 m. There is no record of *O. darwinii* inside the Patos Lagoon estuary (Chao et al. 1985, Garcia et al. 2003), which means that this fish is typically marine.

The Remo flounder is a non-commercial fish that reaches up to 35 cm in total length. It is often caught as by-catch of artisanal and commercial fisheries in coastal waters, less than 40 m deep, throughout its distribution range (Reis et al. 1994, Fabré and Díaz de Astarloa 1996, Dumont and D'Incao 2011). Biological and ecological studies on this species are rare (Fabré and Díaz de Astarloa 1996, Prisco et al 2001); consequently, there is little data to base conservation efforts on. Most studies on *O. darwinii* are reports of by-catch with other target species in fisheries (Segura et al. 2008, Schroeder et al. 2014, Branco et al. 2015, Santos et al. 2016). The lack of bio-ecological information, high frequency as by-catch and the fact that this is an endemic species creates a major concern in terms of species conservation. Here we endeavoured to (1) determine the diet and feeding strategy of juveniles of the Remo flounder in a subtropical sandy beach in southern Brazil and (2) to compare the diet composition and to determine whether it varies with sampling sites, season and fish size.

MATERIAL AND METHODS

Fish were caught from two exposed sandy beaches along the coast of Rio Grande do Sul in southern Brazil, Cassino and Mar Grosso beaches (Fig. 1). Monthly samplings were carried out on three sites at each beach. The sampling sites functioned as spatial replicates and the monthly samplings as temporal replicates. Since there were no significant differences between the environmental variables measured at the three sites in each beach (see Rodrigues and Vieira 2013, Rodrigues et al. 2015), we analysed the samples as a whole, splitting the analysis only by beach.

The fish were collected monthly from August 2009 to July 2010 using two types of beach seine nets: Beach Seine 9 m (BS-9 m) is a multifilament net 9 m in length and 1.5 m in height, 13 mm stretch mesh in the wings and a 5 mm stretch mesh in the centre 3 m section; and the Beach Seine 30 m (BS-30 m) is a net 30 m in length, 1.8 m in height and 12 mm monofilament nylon stretch mesh with a 30 cm inferior bar and 12 mm multifilament stretch mesh. Five hauls using each of the different fishing gear configurations were made in each sampling site. Fish collecting was authorized by the license issued by the "Sistema de Autorização e Informação em Biodiversidade (SISBio/ICMBio/MMA)" of the Brazilian government, under number 24417-1 (authentication code 49915343).

All individuals caught were transported on ice to the laboratory. Each individual was measured (Total Length, mm TL), weighed (g) and dissected to extract the stomach, which was

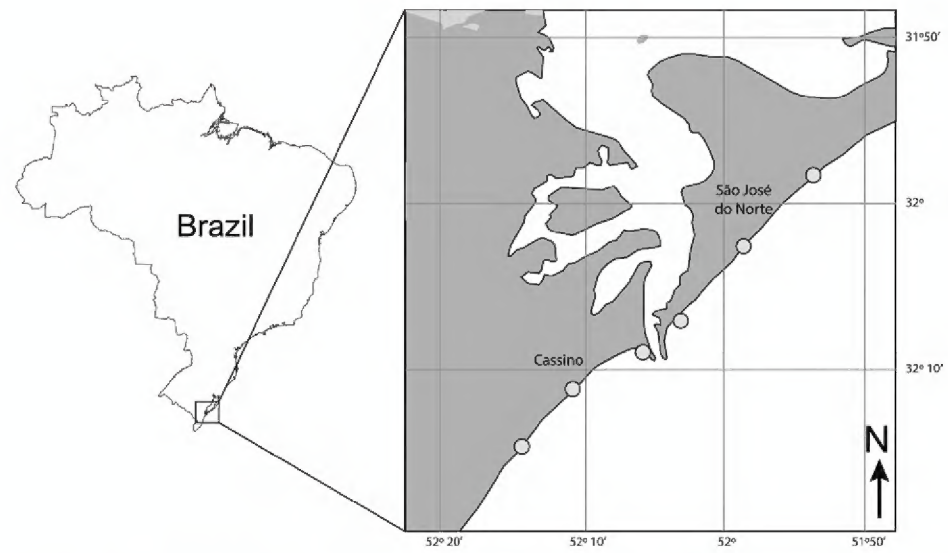


Figure 1. Location of the six sampling sites in the coastline of Rio Grande do Sul, Brazil.

preserved in 10% formaldehyde and stored in 70% alcohol a week later for analysis. Briefly, each stomach was weighed before and after removing all the food items. The food items recovered from each stomach were counted and their volume (0.1 mm^3 precision) was measured. Volume was estimated based on the area occupied by each item and its height (area × height) (e.g. Hellawell and Abel 1971). The food items that were encountered in each stomach analysed were identified to the lowest possible taxonomic level.

A total of 758 Remo flounder flatfish were examined for stomach content analyses. The quantitative analysis of the diet consists of calculating the Coefficient of Vacuity (C_v), which is the percentage of empty stomachs with respect to the total number of stomachs examined (Bouchereau et al. 2009). The food items were quantified using the following parameters: frequency of occurrence (%F; represents the number of non-empty stomachs (in percentage) that a particular food item was found), numerical abundance (%N; total number in percent of a diet item in relation to the total number of items or categories encountered in non-empty stomachs), and volume (%V; that represents the total volume (in percentage) of an particular food item in relation to the total number of food items found in all non-empty stomachs) following Hyslop (1980). The above parameters were subsequently used to calculate the Index of Relative Importance (%IRI; Pinkas et al. 1971, Cortés 1997) as: %IRI = %F * [%N +%V].

To allow a clearer view of the seasonal feeding behaviour of the flatfish throughout the study period, the months were grouped into austral seasons (winter = July to September; spring = October to December; summer = January to March; and autumn = May to June). There were no individuals captured during the austral autumn and early winter (from March to July). To assess the differences in diet associated with the size of the predator (TL mm), individuals of *O. darwinii* were pooled into three size classes: <60 mm; 60–100 mm and >100 mm. These three size classes were chosen arbitrarily and were based on the sizes of the individuals captured by the different fishing gears. The %IRI for each food item was computed for each site and size class.

A one-way ANOVA was used to evaluate the differences in the average number of prey items and prey volume among the austral season (and predefined size classes), and post-hoc differences were evaluated using the Newman-Keuls test ($\alpha = 0.05$). The normality and homogeneity of variance were assessed using Kolmogorov-Smirnov and Cochran tests (Zar 1994), respectively, and the data were log-transformed prior to the analyses to meet the statistical assumptions.

The graphical analysis proposed by Amundsen et al. (1996) was employed to analyse the feeding strategy and to evaluate the contribution of each individual (phenotype) to population niche width. This approach is based on a two-dimensional representation of prey-specific abundance (*Pi*) and %F of the different food items in the diet. The 'prey-specific abundance of prey' ($P_i = (\sum S_i / \sum S_{ti}) \times 100$) takes into account only those predators in which the actual prey (food item) occurs; where P_i is the prey-specific abundance of prey i, S_i is the stomach content (weight) comprising prey i, and S_{ti} is the total stomach content in only those predators with prey i in their stomach (Amundsen et al. 1996). We employed this graphical analysis for both sites.

RESULTS

Oncopterus darwinii occurs in the southwestern Atlantic Ocean from mid-winter (August) to mid-summer (February) and is more frequent between October and January (Fig. 2). Stomach analyses of 325 individuals ranging between 22 to 134 mm TL (77.4 mm ± 19.4 mm) were conducted for samples collected from Cassino, and of 364 individuals ranging between 14 to 123 mm TL (68.6 mm ± 23.2 mm) for samples from Mar Grosso. Sixty-nine stomachs were empty, which corresponds to an annual digestive vacuity of 9.1%.

The non-empty stomachs revealed 17 food items plus remains of organic material (Table 1). Of these, 16 and 11 food items were recorded from stomach analyses of individuals caught from Cassino and Mar Grosso, respectively. Ten food items (plus organic material remains) were found in the stomach contents of fish caught from both beaches. Two prey species (*Bathyporeiapus* sp. and *Emerita brasiliensis* Schmitt, 1935) and one group of Mysida (unidentified) were broadly represented with more than 98% of the contributions in both beaches. *Bathyporeiapus* sp. was responsible for 70% of the food items in Cassino and over 85% in Mar Grosso. In contrast, the contribution of *E. brasiliensis* was greater in Cassino (%IRI = 6.49) than in Mar Grosso (%IRI = 3.95). The contribution of the Mysida group presented a reverse trend (%IRI = 6.53 in Cassino and 21.69 in Mar Grosso) (Table 1). The other food items contributed less than 2% at both beaches.

The coefficient of vacuity was higher during the spring at both beaches (Table 2). In Cassino, the %IRI of *Bathyporeiapus* sp. was higher in all seasons; however, IRI was lower during the spring, when the Mysida group represented 38.58%; the IRI of the Mysida group was also high in winter (%IRI = 17.90) and lower in summer. In contrast, the IRI of *E. brasiliensis* was high only during

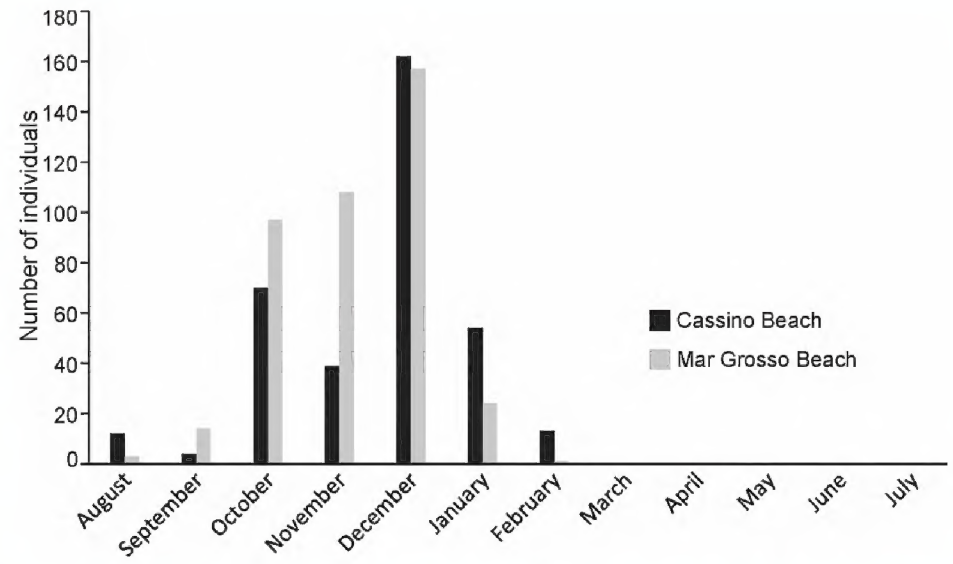
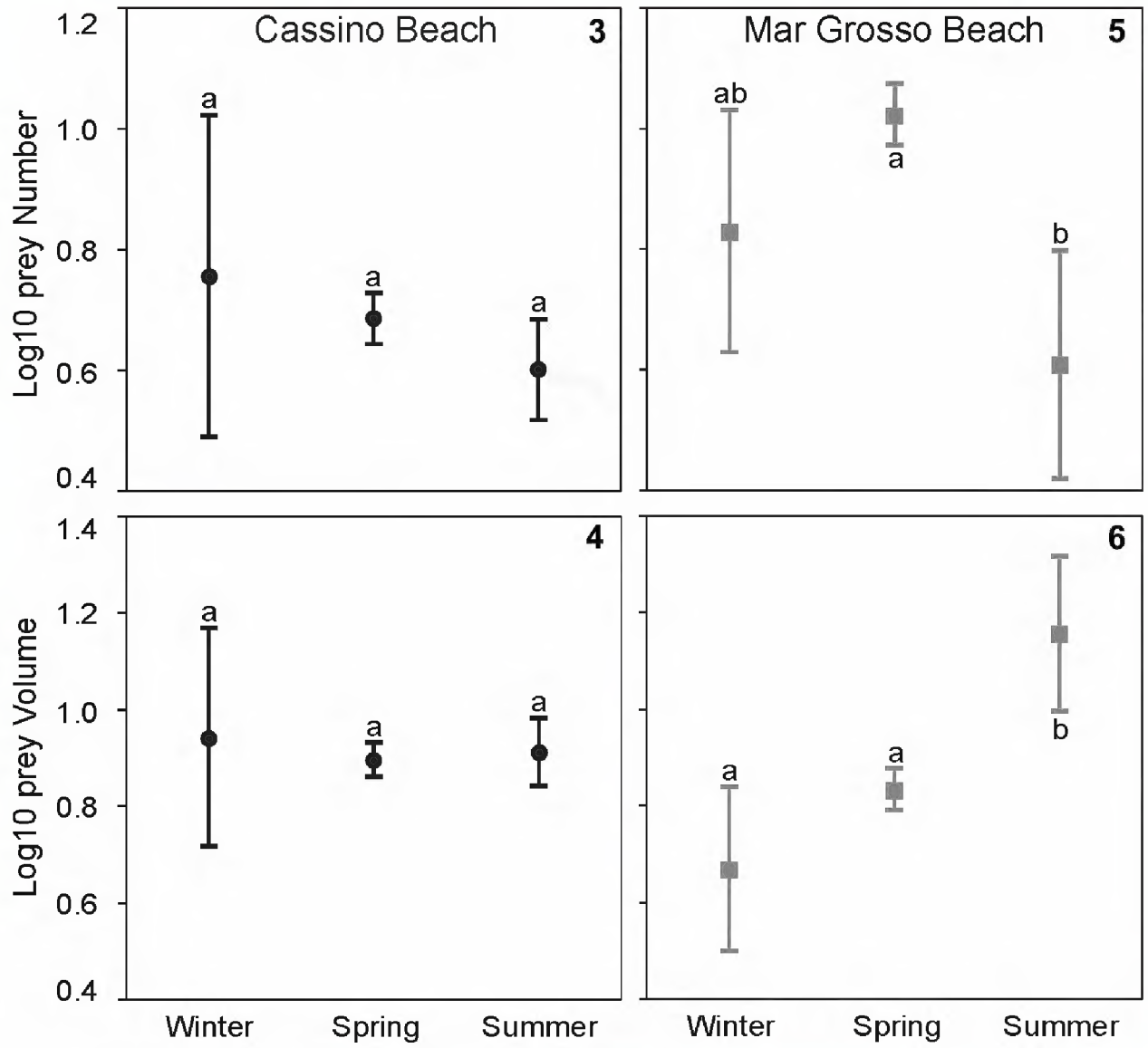


Figure 2. Number of *Oncopterus darwinii* juveniles caught at Cassino (black bars) and Mar Grosso (grey bars) between August 2009 and July 2010.

Table 1. Frequency of occurrence (%F), numerical abundance(%N), volume (%V) and Index of Relative Importance (%IRI) for each prey item, in the gut of juveniles flatfish *Oncopterus darwinii* caught in surf zone of sandy beaches in southern Brazil.

Prey items	Cassino Beach (n = 325)				Mar Grosso Beach (n = 364)			
	%F	%N	%V	%IRI	%F	%N	%V	%IRI
Insecta unidentified	0.27	0.01	0.01	<0.01	–	–	–	–
Bivalvia								
Amarilladesma mactroides	1.61	0.06	0.10	<0.01	3.00	0.05	0.14	0.01
Gastropoda unidentified	0.94	0.02	0.02	<0.01	–	–	–	–
Amphipoda								
Hyperiidae unidentified	7.04	0.50	1.20	0.15	1.28	0.07	0.10	<0.01
Bathyporeiapus bisetosus	87.88	73.13	48.86	73.28	92.21	93.00	52.47	86.76
Phoxocephalopsidae unidentified	12.50	0.85	2.47	0.57	8.89	0.93	0.80	0.17
Decapoda								
Shrimp unidentified	0.72	0.02	0.10	<0.01	–	–	–	–
Crustacea unidentified	6.13	0.23	0.82	0.06	3.53	0.09	0.10	0.01
Pinnotheridae								
Austinixa patagoniensis	1.48	0.04	0.25	<0.01	–	–	–	–
Penaeidae								
Farfantepenaeus paulensis	–	–	–	–	0.19	<0.01	<0.01	<0.01
Hippidae								
Emerita brasiliensis	27.87	2.13	14.05	3.95	18.70	2.68	20.99	6.49
Portunidae unidentified	1.18	0.05	0.12	<0.01	0.19	<0.01	<0.01	<0.01
Callinectes sapidus	4.05	0.13	1.51	0.10	–	–	–	–
Isopoda								
Excirolana armata	8.81	0.41	1.03	0.09	3.58	0.34	0.35	0.02
Chiriscus giambiagiae	5.29	0.30	0.76	0.09	0.38	0.01	0.01	<0.01
Mysida								
Mysida unidentified	56.74	21.93	28.26	21.69	19.01	2.76	24.98	6.53
Actinopterygii								
Menticirrhus spp.	0.27	0.01	0.09	<0.01	–	–	–	–
Organic material remains	1.99	0.11	0.35	0.01	1.47	0.07	0.05	<0.01



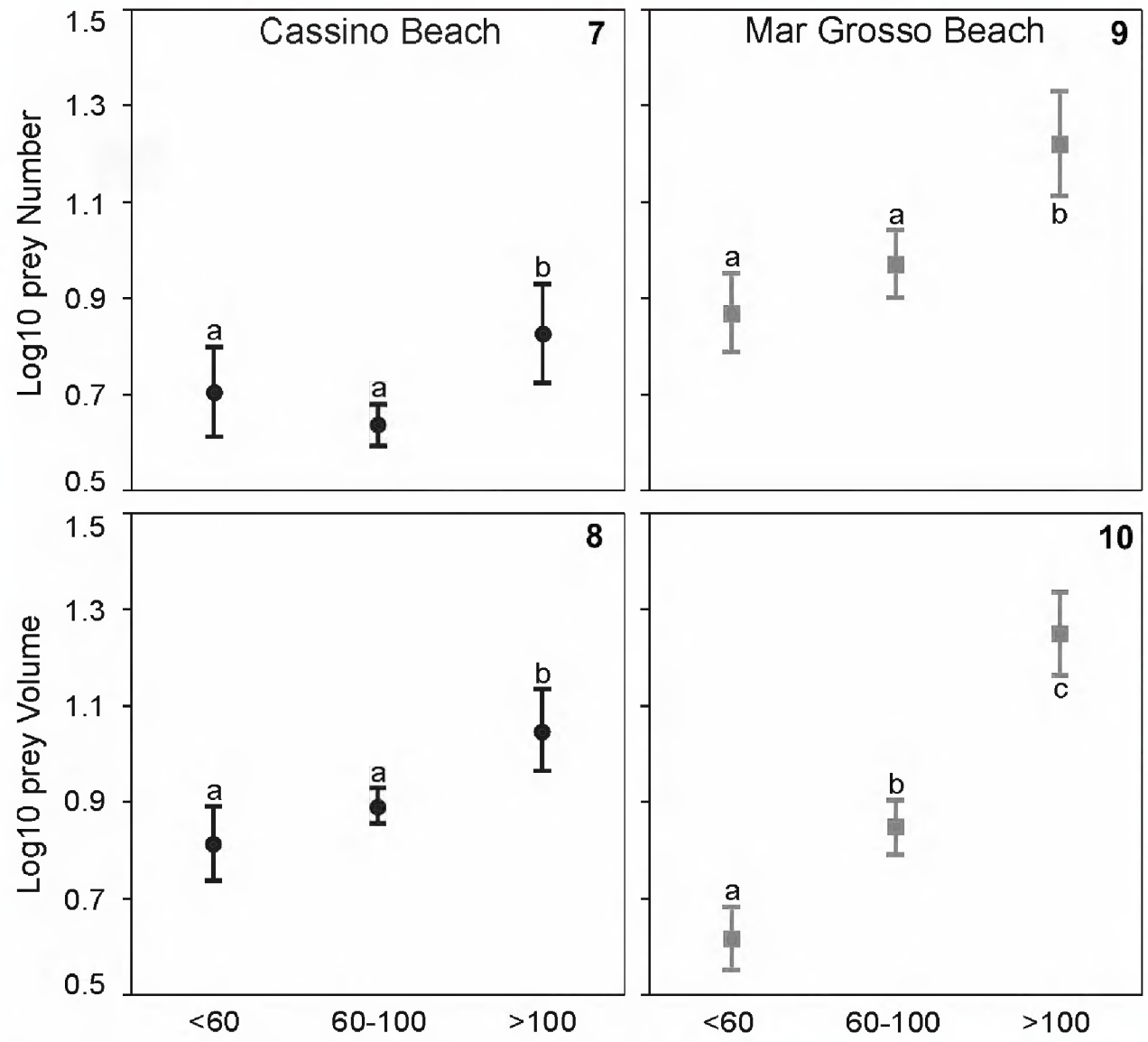
Figures 3–6. Average values (± SD) of prey number and volume in the stomach contents of *Oncopterus darwinii* at Cassino (3–4) and Mar Grosso (5–6), respectively. The data are summarized for three seasons (winter, spring and summer) and shared letters above each box indicate non-significant differences among the seasons.

summer (%IRI = 26.05) (Table 2). In Mar Grosso, *Bathyporeiapus* sp. occurred almost exclusively during the spring (%IRI = 93.02), whereas unidentified Mysida and *E. brasiliensis* had the greatest IRI during winter and summer (%IRI = 45.95 and 55.02, respectively) (Table 2). The number and volume of food items found in the stomachs of flatfish did not differ among the seasons of the year (all ANOVAs $p > 0.05$) for all individuals collected in Cassino. However, in Mar Grosso, the number of food items was greater in spring, with significant difference from the number of food items in summer (ANOVA $p < 0.05$); the volume of food items increased with the seasons, increasing from winter to spring and from spring to summer (ANOVAs $p < 0.05$, Table 2, Figs 3–6).

The percentage of digestive vacuity was greater in the major size classes in both beaches (Table 3). *Bathyporeiapus* sp. was the main food item in the juvenile Remo flounder at both sites; in Cassino, the IRI of this food item was between 70 and 77%, and it was the most important food item for individuals <60 mm (%IRI = 77.06). In Mar Grosso, the IRI of *Bathyporeiapus* sp. was between 85 and 99% with IRI almost 100% for individuals <60 mm (%IRI = 99.04) (Table 3). The Mysida group was constant throughout the immature stages of the Remo flounder from Cassino (%IRI between 20 and 23), whereas in Mar Grosso, this group was important only for individuals between 60–100 mm TL (%IRI = 13.64). Finally, the IRI of *E. brasiliensis* was minor at both sites (Table 3). In relation to the number and the volume of food items found in the stomachs of flatfish, these values increased with the size of the body of *O. darwinii* (all ANOVAs $p < 0.05$, Table 3, Figs 7–10).

Table 2. Variations in Index of Relative Importance of the main items ingested by the juvenile flatfish of the Cassino and Mar Grosso beaches, during a year. Non-empty stomachs (N), Coefficient of Vacuity (Cv), mean number and area (mm^3) of preys (Nm/Am). Do not have any individual collected in Autumn.

Prey items	Cassino Beach			Mar Grosso Beach		
	Winter	Spring	Summer	Winter	Spring	Summer
Bathyporeiapus unidentified	79.84	58.83	72.27	54.04	93.02	36.12
Mysida unidentified	17.90	38.58	0.10	45.95	6.92	<0.01
Emerita brasiliensis	0.71	1.48	26.05	<0.01	0.03	55.02
Others	1.54	1.11	1.58	0.01	0.04	8.86
N	10	252	63	17	323	24
Cv	0.79	2.51	0.53	<0.01	5.15	0.13
Nm/Am	14.4/ 9.83	11.1/ 13.4	8.2/ 13.98	13.3/ 7.57	33.5/ 28.8	6.6/ 34.39



Figures 7–10. Average values (± SD) of prey number and volume in the stomach contents of *Oncopterus darwinii* at Cassino (7–8) and Mar Grosso (9–10), respectively. The data are summarized for three size classes (<60 mm TL, 60–100 mm TL, and >100 mm TL), and shared letters above each box indicate non-significant differences among the size classes.

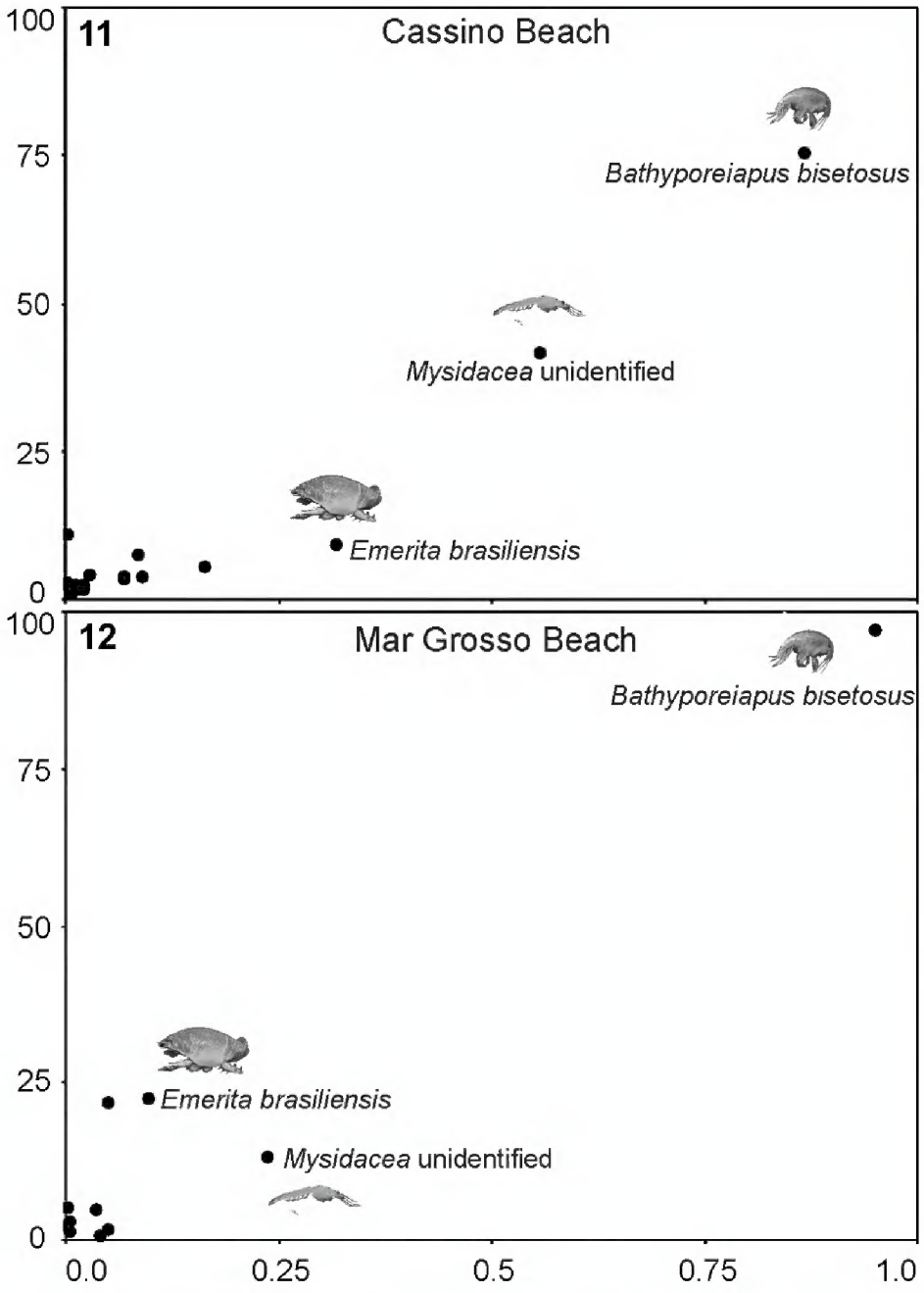
Table 3. Variations in Index of Relative Importance of the main items ingested by the juvenile flatfish of the Cassino and Mar Grosso beaches, along the ontogeny. Non-empty stomachs (N), Coefficient of Vacuity (Cv), mean number and area (mm^3) of preys (Nm/Am). Do not have any individual collected in Autumn.

Prey items	Cassino Beach			Mar Grosso Beach		
	<60	60–100	>100	<60	60–100	>100
Bathyporeiapus unidentified	77.06	70.33	76.01	99.04	85.30	95.24
Mysida unidentified	20.41	21.71	22.82	0.95	13.64	3.87
Emerita brasiliensis	2.04	6.68	0.70	<0.01	1.00	0.67
Others	0.49	1.28	0.48	0.01	0.06	0.22
N	62	219	44	145	163	56
Cv	0.66	0.66	2.51	1.06	0.13	4.09
Nm/Am	10.6/ 9.21	9.6/ 12.75	16.2/ 22.1	17.7/ 6.36	27.4/ 38.5	63.3/ 41.3

According to the Amundsen diagram, the flatfish had a specialist feeding strategy characterized by a single or few prey items, which reflects a predatory population with a narrow niche width (Fig. 11). This pattern was similar in both sites, where *Bathyporeiapus* sp. was the main food item, followed by Mysida group, *E. brasiliensis* and Phoxocephalopsidae unidentified. However, at Mar Grosso, *O. darwinii* was highly specialized in the capture of *Bathyporeiapus* sp. (Fig. 12).

DISCUSSION

The Remo flounder *O. darwinii* is highly abundant in the surf zone of the southern Brazilian sandy beaches in the spring and early summer, and becomes infrequent or scarce during the remainder of the year (Lima and Vieira 2009, Rodrigues and Vieira 2013, Rodrigues et al. 2015). In the extreme southern portion of its distribution (Argentinian waters), Remo flounder populations peak only in summer (Prisco et al. 2001, Jaureguizar et al. 2003). The fact that only juveniles were collected at both beaches and more than 85% of the collected fish were smaller than 100 mm TL indicates that these beaches are used as a nursery ground by this species. We know that juvenile flatfish use the surf zone of sandy beaches during their early life cycle, and when they mature, they move to deeper waters (Beyst et al. 1999, Nissling et al. 2007, Vinagre et al. 2011). In the only study regarding the ecology of *O. darwinii* (Prisco et al. 2001), juveniles were found only in the region near the mouth of Mar



Figures 11–12. Feeding strategy diagram for individuals of *Oncopterus darwinii* caught at both sites: (11) Cassino; (12) Mar Grosso). The prey-specific abundance plotted against the frequency of occurrence of food items for *O. darwinii*.

Chiquita Lagoon (Argentina) and not in the adjacent sandy beaches, as we found.

The low frequency of empty stomachs in our data (9.1%) is consistent with the results of other studies (Beyst et al. 1999, Arrington et al. 2002, Vinagre et al. 2011). The analysis of the percentage of digestive vacuity during the seasons and during the ontogeny revealed that it was higher during the spring and in larger size classes, although in both cases the values were low. This contrasts with previous research that found that digestive vacuity is higher in the winter (Belghyti et al. 1993, de Juan et al. 2007, Teixeira et al. 2010). Our contrasting results are probably due to the fact that in the spring there were more individuals collected. The feeding activity of smaller individuals was higher than the feeding activity of the largest individuals, which had a high frequency of empty stomachs. This feeding behaviour is consistent with other flatfish species (Belghyti et al. 1993, de Juan et al. 2007, Teixeira et al. 2010).

A low diversity of items was found in the digestive tract of *O. darwinii* (16 at Cassino and 11 at Mar Grosso) in our data. The amphipod *Bathyporeiapus* sp. was the most consumed food item at both sites (%IRI = 80), whereas the other two food items (Mysida group and *E. brasiliensis*) were responsible for almost 18% of the IRI. These results reveal that the population of *O. darwinii* had a specialist feeding behaviour, which is characterized by a high consumption of a few items, mainly *Bathyporeiapus* sp. However, some individuals in the population displayed an opportunistic behaviour towards the consumption of certain prey (Mysida group and *E. brasiliensis*). Prisco et al. (2001) also found a high %IRI for gammarid amphipods, which showed the same specialist feeding behaviour that we found in this work. The same pattern was reported for other species of flatfish in South America: *Citharichthys spilopterus* Günther, 1862, *Etropus crossotus* Jordan & Gilbert, 1882 and *Symphurus tessellatus* (Quoy & Gaimard, 1824) (Guedes and Araújo 2008) and *Paralichthys orbignyanus* (Valenciennes, 1839) (Prisco et al. 2001).

No seasonal differences in the diet composition of Remo flounder juveniles were found in the present study (*Bathyporeiapus* sp., *E. brasiliensis* and the Mysida group were the main food items). However, there are variations in the IRI among seasons; this can probably be explained by a decrease in the abundance of the preferred food items throughout the year. Studies made in this region showed a peak occurrence of *Bathyporeiapus* sp. during the spring and a predominance of *E. brasiliensis* during the summer (Neves et al. 2007, 2008, Silva et al. 2008), whereas the representatives of the Mysida group are common throughout the year due to species succession (Montú et al. 1998). Prisco et al. (2001) did not find a seasonal difference in the diet composition of juveniles of *O. darwinii* (either Gammaridae amphipods or Mysida) and the frequency of occurrence of most important food items changed in during the year. When the number and volume of food items were analysed, we found differences only in the individuals caught at Mar Grosso. In this site, the difference occurred in the summer, where prey was in fewer numbers and prey volume was higher. This difference occurred due to the size of *E. brasiliensis* because this food item is larger than the other prey and because *E. brasiliensis* was most abundant in the summer, contributing to more than 50% of the IRI.

Our results were similar for both sites. There were no differences in the diet composition of juveniles of *O. darwinii* throughout its ontogeny, and the same pattern of IRI was maintained. However, a huge variation in prey number and volume found in the stomachs was observed in smaller Remo flounders, with less prey in number and volume than larger flounders. This pattern is common in different species in studies addressing diet composition and variations in ontogeny (Teixeira et al. 2010, Condini et al. 2011) and can be attributed to a combination of factors, for instance a larger mouth, improved ability to handle prey, and faster swim capability.

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